CLAIM AMENDMENTS

1. (Original) A method for quantitatively detecting an antigen which comprises:

a first step of providing an Fab′ antibody having a uniform isoelectric point, said antibody forming an immune complex with an antigen in an analytical sample and being modified by adding an amino acid sequence comprising a charged amino acid residue and by being labeled with a fluorescent dye;

a second step of mixing the Fab′ antibody having a uniform isoelectric point with the analytical sample containing the antigen to obtain a mixture comprising the immune complex;

a third step of separating the mixture by performing electrophoresis in a carrier;

a fourth step of irradiating an excitation light which excites the fluorescent dye to the mixture separated in the third step to cause fluorescence in the immune complex; and

a fifth step of detecting the fluorescence.

2. (Currently Amended) ~~A~~ The method according to claim 1, wherein the amino acid sequence is added adjacent to a C-terminal of an L chain of the Fab′ antibody having a uniform isoelectric point.

3. (Currently Amended) ~~A~~ The method according to claim 1, wherein the fluorescent dye is bound to a cysteine residue which is not involved in binding with an L chain and which exists in an amino acid sequence adjacent to a C-terminal of a CH1 region of the Fab′ antibody having a uniform isoelectric point.

4. (Currently Amended) ~~A~~ The method according to claim 1, wherein the electrophoresis is performed by isoelectric focusing.

5. (Currently Amended) ~~A~~ The method according to claim 1, wherein the electrophoresis is performed by capillary electrophoresis.

6. (Currently Amended) ~~A~~ The method according to claim 1, wherein the Fab′ antibody having a uniform isoelectric point is produced by a method which comprises:

a first step of providing an Fd chain gene encoding a VH region, a CH1 region, and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in an Fab′ antibody;

a second step of site-specifically mutating in the Fd chain gene at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a modified Fd chain gene;

a third step of linking the modified Fd chain gene and an L chain gene encoding an L chain of the Fab′ antibody in the expressible state to obtain a gene expressing a modified Fab′ antibody;

a fourth step of modifying the gene expressing a modified Fab′ antibody to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the L chain to obtain a gene expressing a charge modified Fab′ antibody;

a fifth step of transforming a host cell with the gene expressing a charge modified Fab′ antibody and culturing the resultant transformant to obtain an Fab′ antibody having a uniform isoelectric point, the Fab′ antibody being modified by adding an amino acid sequence comprising a charged amino acid residue adjacent to the C-terminal of the L chain, and by adding an amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal of the CH1 region; and

a sixth step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab′ antibody having a uniform isoelectric point obtained in the fifth step.

7. (Currently Amended) ~~A~~ The method according to claim 1, wherein the Fab′ antibody having a uniform isoelectric point is produced by a method which comprises:

a first step of providing an Fd chain gene encoding a VH region, a CH1 region, and an amino acid sequence which adjoins to a C-terminal of the CH1 region and

comprises a cysteine residue which is not involved in binding with an L chain in an Fab′ antibody;

a second step of site-specifically mutating in the Fd chain gene at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a modified Fd chain gene;

a third step of providing an L chain gene encoding an L chain of the Fab′ antibody;

a fourth step of modifying the L chain gene to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the L chain to obtain a charge modified L chain gene;

a fifth step of linking the modified Fd chain gene and the charge modified L chain gene in the expressible state to obtain a gene expressing a charge modified Fab′ antibody;

a sixth step of transforming a host cell with the gene expressing a charge modified Fab′ antibody and culturing the resultant transformant to obtain an Fab′ antibody having a uniform isoelectric point, the Fab′ antibody being modified by adding an amino acid sequence comprising a charged amino acid residue adjacent to the C-terminal of the L chain, and by adding an amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal of the CH1 region; and

a seventh step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab′ antibody having a uniform isoelectric point obtained in the sixth step.

8. (Currently Amended) ~~A~~ The method according to claim 1, wherein the Fab′ antibody having a uniform isoelectric point is produced by a method which comprises:

a first step of providing an Fd chain gene encoding a VH region, a CH1 region, and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in an Fab′ antibody, and an L chain gene encoding the L chain of the Fab′ antibody;

a second step of linking the Fd chain gene and the L chain gene in the expressible state to obtain a gene expressing an Fab′ antibody;

a third step of modifying the gene expressing an Fab′ antibody to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the L chain, and site-specifically mutating in the gene expressing an Fab′ antibody at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a gene expressing a charge modified Fab′ antibody;

a fourth step of transforming a host cell with the gene expressing a charge modified Fab′ antibody and culturing the resultant transformant to obtain an Fab′ antibody having a uniform isoelectric point, the Fab′ antibody being modified by adding an amino acid sequence comprising a charged amino acid residue adjacent to the C-terminal of the L chain, and by adding an amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal of the CH1 region; and

a fifth step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab′ antibody having a uniform isoelectric point obtained in the fourth step.

9. (Currently Amended) ~~A~~ The method according to claim 1, wherein the Fab′ antibody having a uniform isoelectric point is produced by a method which comprises:

a first step of providing a CH1 gene encoding a CH1 region and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in a first Fab′ antibody, and a CL gene encoding a CL region of the first Fab′ antibody;

a second step of site-specifically mutating in the CH1 gene at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a modified CH1 gene;

a third step of cutting the modified CH1 gene with a restriction enzyme to obtain a gene fragment encoding the CH1 region;

a fourth step of providing a VH gene encoding a VH region of a second Fab′ antibody and a VL gene encoding a VL region of the second Fab′ antibody;

a fifth step of linking the gene fragment, the CL gene, the VH gene and the VL gene in the expressible state to obtain a gene expressing a modified Fab′ antibody;

a sixth step of modifying the gene expressing a modified Fab′ antibody to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the CL region to obtain a gene expressing a charge modified Fab′ antibody;

a seventh step of transforming a host cell with the gene expressing a charge modified Fab′ antibody and culturing the resultant transformant to obtain an Fab′ antibody having a uniform isoelectric point, the Fab′ antibody being modified by adding an amino acid sequence comprising a charged amino acid residue adjacent to the C-terminal of the L chain, and by adding an amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal of the CH1 region; and

a eighth step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab′ antibody having a uniform isoelectric point obtained in the seventh step.

10. (Currently Amended) ~~A~~ The method according to claim 1, wherein the Fab′ antibody having a uniform isoelectric point is produced by a method which comprises:

a first step of providing a CH1 gene encoding a CH1 region and an amino acid sequence which adjoins to a C-terminal of the CH1 region and comprises a cysteine residue which is not involved in binding with an L chain in a first Fab′ antibody, and a CL gene encoding a CL region of the first Fab′ antibody;

a second step of site-specifically mutating in the CH1 gene at least one codon encoding an amide group-containing amino acid residue in the CH1 region, into a codon encoding an amide group-non-containing amino acid residue except for cysteine to obtain a modified CH1 gene;

a third step of cutting the modified CH1 gene with a restriction enzyme to obtain a gene fragment encoding the CH1 region;

a fourth step of modifying the CL gene to express an amino acid sequence comprising a charged amino acid residue adjacent to a C-terminal of the CL region to obtain a charge modified CL gene;

a fifth step of providing a VH gene encoding a VH region of the second Fab′ antibody and a VL gene encoding a VL region of the second Fab′ antibody;

a sixth step of linking the gene fragment, the charge modified CL gene, the VH gene and the VL gene in the expressible state to obtain a gene expressing a charge modified Fab′ antibody;

a seventh step of transforming a host cell with the gene expressing a charge modified Fab′ antibody and culturing the resultant transformant to obtain an Fab′ antibody having a uniform isoelectric point, the Fab′ antibody being modified by adding an amino acid sequence comprising a charged amino acid residue adjacent to the C-terminal of the L chain, and by adding an amino acid sequence comprising a cysteine residue which is not involved in binding with an L chain adjacent to the C-terminal of the CH1 region; and

a eighth step of binding a fluorescent dye to the cysteine residue which is not involved in binding with an L chain in the Fab′ antibody having a uniform isoelectric point obtained in the seventh step.

11. (New) A modified Fab′ antibody comprising an Fd chain and an L chain, the Fd chain comprising a CH1 region and the L chain comprising a CL region, wherein at least one of amide side chain containing amino acid residues for the CH1 region and the CL region is substituted by a non-amide side chain containing amino acid residue except for cysteine.

12. (New) The modified Fab′ antibody according to claim 11, wherein the Fd chain contains a polypeptide added adjacently to a C-terminal of the CH1 region and the polypeptide contains a cysteine residue which is not involved in binding with the L chain.

13. (New) The modified Fab′ antibody according to claim 12, wherein the cysteine residue is bound to a fluorescent dye.

14. (New) The modified Fab′ antibody according to claim 11, wherein the Fd chain or the L chain contains a polypeptide added adjacently to a C-terminal of either chain and the polypeptide contains a charged amino acid residue.

15. (New) A polynucleotide encoding a modified Fab′ antibody which comprises an Fd chain and an L chain with the Fd chain comprising a CH1 region and the L chain comprising a CL region,

the polynucleotide comprising an Fd chain coding region and an L chain coding region linked therebetween in a manner that will allow the expression of the modified Fab′ antibody,

wherein at least one of codons encoding amide side chain containing amino acid residues for the CH1 region and the CL region is specifically mutated into a codon encoding a non-amide side chain containing amino acid residue except for cysteine.

16. (New) The polynucleotide according to claim 15, wherein the Fd chain coding region contains a polynucleotide encoding a polypeptide added adjacently to a C-terminal of the CH1 region containing a cysteine residue which is not involved in binding with the L chain.

17. (New) The polynucleotide according to claim 16, wherein the Fd chain coding region contains a polynucleotide encoding a polypeptide set forth in SEQ ID NO:9 in the Sequence Listing.

18. (New) The polynucleotide according to claim 15, wherein the Fd chain coding region or the L chain coding region contains a polynucleotide encoding a polypeptide added adjacently to a C-terminal of either chain and containing a charged amino acid.

19. (New) The nucleotide according to claim 18, wherein the L chain coding region contains a polynucleotide encoding a polypeptide set forth in SEQ ID NO:12 in the Sequence Listing.

20. (New) An expression vector containing the polynucleotide according to claim 15.

21. (New) A transformant containing the expression vector according to claim 20.